Role of L-type Ca²⁺ channel in PACAP-induced adrenal catecholamine release in vivo

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Geng, Guoju, Rania Gaspo, Fethi Trabelsi, and Nobuharu Yamaguchi. Role of L-type Ca²⁺ channel in PACAP-induced adrenal catecholamine release in vivo. Am. J. Physiol. Regul. Integrative Comp. Physiol. 273 (Regulatory Integrative Comp. Physiol. 42): R1339-R1345, 1997.—The aim of the present study was to investigate whether the dihydropyridine-sensitive L-type Ca²⁺ channel is operative in adrenal catecholamine (CA) secretion induced by a novel neuropeptide, pituitary adenylate cyclase-activating polypeptide (PACAP), in anesthetized dogs. Plasma CA concentrations in adrenal venous and aortic blood were determined by a high-performance liquid chromatography method. All drugs tested were locally infused into the left adrenal gland via the left adrenolumbar artery. PACAP, with the isoform consisting of 27 (PACAP-27) and 38 (PACAP-38) amino acid residues, significantly increased CA output in a dose-dependent manner, with doses ranging from 5 to 500 ng and 7 to 700 ng, respectively. However, the amplitude of epinephrine response to PACAP-27 was three times greater than that obtained with PACAP-38 at the highest dose tested. In a separate group, a single dose of PACAP-27 (50 ng) induced highly reproducible CA responses when the same dose was repeated with an interval of 35 min. In dogs treated with nifedipine (50 μg), 5 min before the second administration of PACAP-27, the net CA response was significantly inhibited by ∼50% compared with that obtained in the presence of vehicle. A similar CA response to BAY K 8644 (5 μg) was completely abolished by the same dose of nifedipine. The present results indicate that both PACAP-27 and PACAP-38 have the direct secretagogue effect on the adrenal medulla in vivo and that CA responses to PACAP-27 were greater than those observed with PACAP-38 at equivalent mol doses. The study suggests that the dihydropyridine-sensitive L-type Ca²⁺ channel is functionally involved in PACAP-induced adrenal CA secretion in the canine adrenal medulla in vivo.

PITUITARY ADENYLATE cyclase-activating polypeptide-27; pituitary adenylate cyclase-activating polypeptide-38; BAY K 8644; nifedipine; medullary secretion; dogs

PITUITARY ADENYLATE cyclase-activating polypeptide (PACAP) is a novel member of the family of vasoactive intestinal polypeptide (VIP). This biologically active neuropeptide exists in two forms, PACAP-38 and PACAP-27, with 38 amino acid residues and the NH₂-terminal-amidated 27 residues, respectively, originally isolated from ovine hypothalamus (16, 18). The structure of PACAP-27 has been shown to be 68% homologous to VIP in their amino acid sequences (10, 18). PACAP is widely distributed in the central nervous system and peripheral organs. The adrenal gland is the one that contains a high concentration of PACAP (2). PACAP is a very potent secretagogue for catecholamines in cultured chromaffin cells from the rat and pig as well as in the isolated, perfused rat adrenal gland (11, 13, 25). PACAP has been postulated to play a role of noncholinergic neurotransmitter in the rat adrenal medulla (24, 26), but its local direct effect on the adrenal gland has not convincingly been demonstrated in other in vivo models. On the other hand, it has been shown that, in cultured porcine chromaffin cells, PACAP-induced catecholamine release was inhibited by L-type Ca²⁺ channel blockers (13), suggesting that PACAP increases Ca²⁺ influx into chromaffin cells and thereby induces the release of catecholamines. Furthermore, it has been demonstrated that the release of adrenal catecholamine depends on the influx of Ca²⁺ into the intracellular compartment of chromaffin cells through voltage-dependent Ca²⁺ channels (4, 15). The dihydropyridine-sensitive L-type Ca²⁺ channel has thus been locally implicated in adrenal catecholamine secretion induced by angiotensin II and endothelin-1 under in vivo conditions (17, 28). However, the potential involvement of L-type Ca²⁺ channel in PACAP-induced catecholamine release still remains unknown in vivo. Therefore, the specific aims of the present study were to investigate whether PACAP possesses the direct secretagogue effect in vivo, and the dihydropyridine-sensitive L-type Ca²⁺ channel is functionally involved in PACAP-induced catecholamine secretion in the canine adrenal gland under in vivo conditions.

METHODS

General preparation of animals. Adult mongrel dogs (27.0 ± 0.8 kg, n = 39), fasted overnight but allowed free access to water, were anesthetized with pentobarbital sodium (30 mg/kg iv, followed by 4 mg/kg as needed; MTC Pharmaceuticals, Cambridge, ON). Respiration was controlled through an endotracheal tube, with room air delivered by a respirator (model 607; Harvard, South Natick, MA). Body temperature of each dog was monitored and kept constant at 37.5 ± 0.5°C by a thermoregulator (model 74; Yellow Springs Instruments, Yellow Springs, OH) connected to a heating pad throughout the experiment. Physiological saline was slowly administered intravenously during the whole period of the experiment to prevent dehydration. The pH of physiological saline was adjusted between 7.35 and 7.45 immediately before use. Both femoral arteries were cannulated; the right femoral artery was used to measure aortic pressure through a catheter, the tip of which was placed at the level of abdominal aorta, and the left femoral artery to obtain aortic blood samples.

Preparation of local intra-arterial drug infusion into left adrenal gland. The experimental model used in this study has been reported elsewhere in full detail (27). Briefly, after a median laparotomy and a left flank incision, the left adrenolumbar artery was dissected free from the surrounding tissues and cannulated in a retrograde manner, so that the tip of catheter (PE-90) was placed either close to or underneath the gland. The volume of this catheter was fixed at 0.5 ml. All visible branches arising from the adrenolumbar artery to-
ward the outside of the gland were ligated to prevent undesired drug diffusion into the systemic circulation. The catheter was connected to an infusion pump (model 1140–001, Harvard).

Preparation of extracorporeal adrenal venous circuit. A specially shaped catheter (PE-240) was inserted into the adrenal vein through the left femoral vein. The volume of this catheter was fixed to be 1.5 ml. The catheter was tied at the adrenodoabdominal vena caval junction to prevent dilution of adrenal venous blood with abdominal vena caval blood. The left adrenolumbar vein distal to the gland was ligated to obtain actual adrenal venous blood. Adrenal venous blood from the left gland was drained into a small reservoir. The blood in the reservoir was connected to a perfusion pump (Masterflex 7016–52; Cole-Parmer Instrument, Chicago, IL) to return adrenal venous blood through a catheter inserted into the right femoral vein at a rate of stabilized initial venous blood flow (27). After all surgical procedures were completed, heparin sodium (200 U/kg iv) was administered, followed by 100 U/kg every hour thereafter. The dog was then allowed a stabilization period of –60 min.

Measured parameters. Aortic blood pressure and heart rate measured from the body surface electrocardiogram were monitored with a polygraph system (model RM-6000; Nihon-Kohden, Tokyo, Japan). Blood samples for plasma catecholamine assays were collected in ice-cooled test tubes. Left adrenal venous blood flow was determined by a gravimetric method, and hematocrit with microhematocrit capillary tubes after 10 min of centrifugation. Aliquots of 1.5 ml of aortic and adrenal venous blood were transferred to chilled centrifuge tubes containing 30 µl of preservative solution (pH 6.5) consisting of ethylene glycol-bis(l-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (95 mg/ml) and glutathione (60 mg/ml). Blood samples for catecholamine measurements were immediately centrifuged at 4°C for 5 min at 15,800 g (model 5402; Centrifuge Eppendorf, Eppendorf, Germany). Plasma was stored at –80°C until the assay was carried out within 2 wk. Plasma epinephrine and norepinephrine concentrations were determined by an electrochemical detector (model 5200; ESA Coulomet II Multi-Electrode Detector, Bedford, MA) coupled with a high-performance liquid chromatographic system (Gilson, Villiers-le-Bel, France) according to the method previously reported from our laboratory (27). At the end of each experiment, the left adrenal gland was removed and weighed. The net catecholamine output was calculated as follows: net output of adrenal catecholamines (ng·min⁻¹·g⁻¹) = ([CA]ADV – [CA]AO) × BFADV × (1 – HtADV)/wet weight of adrenal gland, where [CA]ADV is plasma catecholamine concentration in adrenal venous blood, [CA]AO is circulating catecholamine concentration in aortic blood, BFADV is adrenal venous blood flow, and HtADV is adrenal venous hematocrit. The amount of catecholamine released during the first 5 min after PACAP-27 was obtained from an area under the curve of net catecholamine output, and the data were expressed as nanogram per gram wet weight of the gland.

Experimental groups and drugs. The present study was carried out in five separate groups: the first two groups served to compare local effects of PACAP-27 (molecular weight = 3147.6; n = 11) and PACAP-38 (molecular weight = 4534.3; n = 7) (Sigma Chemical, St. Louis, MO) on the adrenal medulla within a dose range from 5 to 500 ng or 7 to 700 ng, respectively; the third group (n = 8), serving as the control group, was to ensure the reproducibility of the adrenal catecholamine responses to repeated single doses of PACAP-27 (50 ng) in the presence of vehicle (6% ethanol prepared with saline); the fourth (n = 9) and fifth (n = 4) groups served to test the effect of nifedipine (50 µg) (Sigma Chemical) on the adrenal catecholamine response induced by PACAP-27 (50 ng) and BAY K 8644 (5 µg) (Calbiochem, La Jolla, CA), respectively. The doses of nifedipine and BAY K 8644 were selected based on our recent observations that nifedipine at the dose used in the present study completely blocked the adrenal catecholamine response to BAY K 8644 in a similar experimental setup (17). The dose of PACAP-27 was selected according to the present dose-response experiments. PACAP-27 and PACAP-38 were dissolved in saline. Nifedipine and BAY K 8644 were first dissolved in ethanol and then diluted with saline to the desired concentration; the final solution contained 6% ethanol.

Experimental protocols. In the first two separate groups in which the dose dependency was tested, dogs received, with an interval of 30 min, three different doses of either PACAP-27 (5, 50, and 500 ng) or PACAP-38 (7, 70, and 700 ng), with the same molar basis (3.2, 32, and 320 nM) for both drugs. One minute after the initial control sample was taken, the first dose of either drug was infused into the left adrenolumbar artery at a rate of 0.5 ml/min. The net infusion period was fixed to be 1 min. The dead volume of the adrenal arterial (0.5 ml) and venous (1.5 ml) catheter was taken into account in relation to the infusion rate and adrenal venous blood flow, respectively. Blood collections were made at 1, 2, 3, 5, 10, 15, 20, and 30 min after the onset of net infusion. The sample obtained 30 min after the drug infusion served as control for the next response. The procedure was repeated in a similar way for the next two higher doses. Two additional samples were collected 105 and 120 min after the third dose to ensure the stability of basal catecholamine secretion.

In the third group receiving the vehicle and PACAP-27, immediately after the initial control sample was taken, the vehicle was infused into the left adrenolumbar artery in a similar way as described above. Then, the second control sample was taken 5 min after the vehicle administration. PACAP-27 (50 ng) was similarly administered, and blood samples were collected at 1, 2, 3, 5, 10, 15, and 30 min after the infusion of PACAP-27. As in the case of the first group, the sample obtained 30 min after PACAP-27 served as control for the next procedure. Then, the second vehicle was administered, followed by the same procedures as those after the first vehicle administration.

In the fourth and fifth group, the effects of PACAP-27 (50 ng) and BAY K 8644 (5 µg) were tested in the presence of nifedipine (50 µg), respectively. The experimental protocol was exactly the same as that described for the third group, with the exception that the second vehicle was replaced with nifedipine administration.

All surgeries and experimental procedures were carried out while dogs were under full surgical anesthesia. All experiments were acute, terminal procedures. At the end of the experimental protocols, each animal used in this study was euthanized following an intravenous overdose of pentobarbital sodium without the animal regaining consciousness.

Statistical analyses. The statistical evaluations and the calculations of an area under the curve were carried out using a statistical software package (SigmaStat and SigmaPlot for Windows, Version 10.0; Jandel Scientific, San Rafael, CA). Differences within subjects observed over a given experimental period were evaluated by an analysis of variance for repeated measures followed by multiple comparisons with one control using the Dunnett’s method (Figs. 1, 3, 5, and 6). Comparisons of catecholamine responses to PACAP-27 before and after nifedipine administration were made using the paired t-test (Fig. 4). Comparisons between different groups were conducted by the use of one-way analysis of variance followed by the Bonferroni’s test (Figs. 2 and 7). The results...
are expressed as mean ± SE, and P < 0.05 was considered statistically significant.

**RESULTS**

Effect of PACAP-27 and PACAP-38 on adrenal catecholamine output. After local infusion of either PACAP-27 or PACAP-38, both adrenal venous catecholamine concentration and blood flow increased without significantly affecting circulating catecholamine concentration in aortic blood. Consequently, the net output of adrenal epinephrine and norepinephrine significantly increased in a dose-dependent manner in response to various doses of PACAP-27 ranging from 5 to 500 ng (Fig. 1). The response was rapid, and the peak response was usually observed during the infusion (Fig. 1). After the cessation of the infusion, the increased catecholamine output returned towards the corresponding pre-infusion control levels within ~5, ~10, and ~15 min following the dose of 5, 50, and 500 ng, respectively (Fig. 1). Thus the duration of action also increased in a dose-dependent manner. Similar dose-related responses were observed with PACAP-38, but the maximum responses were significantly smaller than those obtained with PACAP-27 (Fig. 2). Mean aortic pressure and heart rate did not significantly change following the local administration of either peptide at any doses tested.

Catecholamine responses to PACAP-27 in the absence or presence of nifedipine. In the control group receiving vehicle, adrenal catecholamine output significantly increased in response to PACAP-27 with a dose of 50 ng. The increases in the output of both epinephrine and norepinephrine were highly reproducible on the second administration of PACAP-27 with the same dose given at an interval of 35 min (Fig. 3). The increased catecholamine output remained significantly higher than the control levels up to at least 5 min after the administration of PACAP-27 (Fig. 3). The quantity of both epinephrine and norepinephrine released during the first 5 min after the onset of PACAP-27 infusion remained unchanged on the second infusion in the vehicle control group (Fig. 4A).

In the group receiving nifedipine (50 µg), the maximum catecholamine response to PACAP-27 observed in the presence of nifedipine was slightly attenuated by ~30% (P = 0.02 and 0.16 for epinephrine and norepinephrine, respectively) as compared with the control response (Fig. 5). In addition, the catecholamine output remained significantly elevated only for 2 min in the presence of nifedipine (Fig. 5). Consequently, the quantity of catecholamines released during the first 5 min after the administration of PACAP-27 was significantly reduced by ~50% in the presence of nifedipine (Fig. 4B). This reduction in PACAP-27-induced catecholamine secretion was significantly different from the value observed in the vehicle control group (Fig. 7). By contrast, the increase in catecholamine output similarly observed in response to BAY K 8644 was almost completely blocked by nifedipine (Figs. 6 and 7).

**DISCUSSION**

The present study demonstrates that the local administration of either PACAP-27 or PACAP-38 into the left adrenal gland of the rat elicits a dose-dependent increase in adrenal catecholamine output.
adrenolumbar artery resulted in a significant increase in adrenal catecholamine secretion in a dose-dependent manner without any significant systemic effect in anesthetized dogs. The study also shows that the amplitude of catecholamine response to PACAP-27 was at least three times greater than that to PACAP-38 at the highest dose tested in the dog model in vivo. The results suggest that the dihydropyridine-sensitive L-type Ca\(^{2+}\) channel is functionally involved in mechanisms regulating adrenal catecholamine secretion induced by PACAP-27.

Because PACAP has been originally isolated from ovine hypothalamic tissues, it has been suggested that PACAP may play distinct roles as a neurotransmitter, neuromodulator, or neurotropic factor in the central nervous system (1). In the peripheral nervous system, PACAP has been proposed to be a noncholinergic neurotransmitter controlling catecholamine secretion in the rat adrenal medulla (24). PACAP has been shown to be a potent secretagogue in various studies in vitro, including the isolated, perfused rat adrenal gland (11) and cultured chromaffin cells obtained from rat (25), frog (29), and porcine adrenal glands (13). More recently, it has been shown that PACAP increased catecholamine secretion from the rat adrenal in vivo (26). In concordance with those previous studies, the present observations are compatible with the view that PACAP may be functionally involved in local regulation of adrenal catecholamine secretion as a potent modulator or secretagogue in anesthetized dogs. Furthermore, we observed in the present study that mean aortic pressure, heart rate, and circulating catecholamine levels in aortic blood did not significantly change even during the infusion of either PACAP-27 or PACAP-38 at their highest dose tested. In this context, it is of interest to note that, in anesthetized dogs, intravenous injection of PACAP-27 resulted in either hypotension or hypertension associated with tachycardia or bradycardia, respectively, depending on the doses administered (12). The latter observations suggest that PACAP may affect the sympathoadrenal activity through cardiovascular reflexes. However, the results of the present study are consistent with the view that the increasing effect of PACAP on catecholamine secretion resulted from its direct action on the adrenal medulla and not from secondary factors such as a reflex-induced increase in sympathetic outflow.

Although adrenal chromaffin cells possess not only L-type but also N-, P-, and Q-type voltage-dependant Ca\(^{2+}\) channels (7), it is well accepted that the L-type Ca\(^{2+}\) channel is a major route of Ca\(^{2+}\) entry (15). In the present study, BAY K 8644, a dihydropyridine L-type Ca\(^{2+}\) channel activator, significantly increased catecholamine secretion. This BAY K 8644-induced catecholamine release was almost completely diminished by nifedipine, clearly indicating the specific antagonism at the level of the dihydropyridine-sensitive L-type Ca\(^{2+}\) channel. With respect to the functional existence of an L-type Ca\(^{2+}\) channel in the dog adrenal medulla in vivo, we have recently demonstrated that the increase in

Fig. 3. Adrenal epinephrine (A) and norepinephrine (B) output in response to repeated administration of PACAP-27 (50 ng) at 0 and 35 min in the vehicle control group. First and second vehicle were administered at -5 and 30 min, respectively. Shaded circles represent control value observed immediately before the vehicle administration. *P < 0.05 vs. corresponding control value indicated by an open circle.

Fig. 4. Net amount of epinephrine (A) and norepinephrine (B) released during the first 5 min after the onset of PACAP-27 (50 ng) infusion in the vehicle (VH) control group (A) and in group receiving nifedipine (Nif, 50 µg; B). *P < 0.05 vs. corresponding vehicle control value. Original data are shown in Figs. 3 and 5.
adrenal catecholamine output induced by BAY K 8644 was significantly inhibited in the presence of nifedipine in a dose-dependent manner under conditions similar to those of the present study (17). These observations are compatible with the view that the dihydropyridine-sensitive L-type Ca\textsuperscript{2+} channel is operative in the local regulation of adrenal catecholamine secretion not only in vitro (4) but also under in vivo conditions (9, 17, 28).

It has been debatable whether the dihydropyridine-sensitive L-type Ca\textsuperscript{2+} channel is functionally involved in PACAP-induced adrenal catecholamine secretion. It has been shown that, in cultured porcine chromaffin cells, catecholamine release induced by PACAP resulted, most probably, from an increase in Ca\textsuperscript{2+} influx by selectively activating a voltage-dependent L-type Ca\textsuperscript{2+} channel, but not N-, P-, or Q-type channels (13). More recently, the whole cell patch-clamp technique revealed that, in bovine adrenal chromaffin cells, PACAP causes both Ca\textsuperscript{2+} release, mainly from caffeine-sensitive Ca\textsuperscript{2+} stores, and Ca\textsuperscript{2+} influx through the dihydropyridine-sensitive L-type Ca\textsuperscript{2+} channel (23). In cultured rat chromaffin cells, however, nifedipine had no effect on PACAP-induced catecholamine secretion (20).

These previous in vitro studies imply the existence of species difference with respect to mechanisms involved in PACAP-induced adrenal catecholamine secretion. In the present study, the maximum increase in catecholamine response to PACAP-27, usually observed during the first minute of infusion, was inhibited by ~30% in the presence of nifedipine, whereas the quan-
tity of catecholamines released during the first 5 min was diminished by ~50%. The lack of more complete inhibition of the PACAP-27-induced response, particularly of the initial rapid increase in catecholamine output, may be due to the dose of nifedipine that was insufficient. This is, however, unlikely to be the case, because the similar catecholamine response to BAY K 8644 was abolished in the presence of nifedipine with the same dose used for the experiments with PACAP-27 in the present as well as in our previous study (17). Furthermore, we have shown that a small dose of nifedipine (one-tenth that used in the present study) markedly attenuated by ~75% the initial steep catecholamine response to endothelin-1, the extent of which was similar to that induced by PACAP-27, in the same dog model used in the present study (28). In addition, the rapid increase in catecholamine response to angiotensin II was also significantly inhibited by ~65% in the presence of nifedipine under conditions similar to those of the present study (17). Taken together, the present findings are compatible with the hypothesis that the Ca\(^{2+}\) entry via the dihydropyridine-sensitive L-type Ca\(^{2+}\) channel may be involved, but play only a minor role, in initiating the exocytotic release of catecholamines induced by PACAP-27 in the canine adrenal medulla in vivo.

It may be of further interest that the PACAP-27-induced increase in catecholamine output remained significantly elevated at least for 5 min, whereas it remained only for 2 min in the presence of nifedipine. It is, therefore, likely that the duration of action of PACAP-27 was significantly shortened by nifedipine. This observation also suggests that the dihydropyridine-sensitive L-type Ca\(^{2+}\) channel is functionally involved in PACAP-27-induced adrenal catecholamine secretion, but its contribution to the initial rapid Ca\(^{2+}\) entry may not be as important as observed in catecholamine release induced by either endothelin-1 (28) or angiotensin II (17). In this context, we have recently observed that VIP-induced adrenal catecholamine secretion remained unchanged in the presence of nifedipine at the same dose used in the present study (8). The latter finding is compatible with the view that the dihydropyridine-sensitive L-type Ca\(^{2+}\) channel may play only a minor role in PACAP-27-induced catecholamine secretion, because PACAP-27 is 68% homologous to VIP in their amino acid sequences and, therefore, shares mechanisms mediated by adenosine 3',5'-cyclic monophosphate (cAMP) (10, 18). Indeed, it has been postulated that, in rat chromaffin cells, PACAP-induced catecholamine release could be initiated by a nifedipine-resistant, cAMP-mediated Ca\(^{2+}\) influx (20).

In conclusion, PACAP is a potent adrenomedullary secretagogue in anesthetized dogs. Both PACAP-27 and PACAP-38 directly stimulate the medulla, resulting in an increase in catecholamine release in a dose-dependent manner. It is most likely that the dihydropyridine-sensitive L-type Ca\(^{2+}\) channel is functionally involved in mechanisms controlling catecholamine secretion induced by PACAP-27.

**Perspectives**

It has been shown that intravenous administration of PACAP produced an increase in systemic blood pressure that was blocked by an \(\alpha\)-adrenoceptor antagonist, phentolamine, in anesthetized dogs (21) and cats (5) as well as in adrenalectomized cats (5). Furthermore, PACAP has been shown to exert a central pressor action by increasing sympathetic outflow in anesthetized dogs (22). These observations strongly suggest that PACAP may play important roles in hormonal and neural control of cardiovascular functions. On the other hand, nifedipine and other similar L-type Ca\(^{2+}\) channel blockers are commonly used in the treatment of cardiovascular diseases such as hypertension and cardiac arrhythmias, which may frequently involve sympathoadrenal dysfunctions (6, 19). In this context, the present findings may contribute to a better comprehension of mechanisms of action of PACAP and its interaction with the L-type Ca\(^{2+}\) channel in the sympathoadrenal system under certain pathophysiological conditions.

With respect to the potential interaction of PACAP with the peripheral autonomic nervous system, it has been shown that PACAP elicited sustained release of catecholamines in cultured superior cervical ganglion (3). In cultured porcine adrenal medullary chromaffin cells, PACAP has also been shown to stimulate sustained catecholamine production resulting from both cAMP- and protein kinase C-dependent activations of tyrosine hydroxylase and dopamine-β-hydroxylase (14). Furthermore, PACAP-27 potentiated the cardiac slowing induced by vagal stimulation, while having no effect on the cardiac response to sympathetic stimulation in anesthetized dogs (21). PACAP-38 has similarly been shown to activate cardiac parasympathetic nerves in the isolated, blood-perfused dog heart (30). These observations suggest that PACAP may facilitate cholinergic, rather than adrenergic, neuroeffector transmission. Our recent study indicated that splanchnic nerve stimulation-induced catecholamine secretion was significantly enhanced in the presence of PACAP-27 in the canine adrenal gland in vivo (S. Lamouche, D. Martinneau and N. Yamaguchi, unpublished observation). The latter observation is compatible with the view that PACAP facilitates the cholinergic neurotransmission in the adrenal medulla. However, the functional implication of mechanisms mediated either by the specific presynaptic PACAP receptors or by nicotinic and/or muscarinic postsynaptic receptors remains to be elucidated.

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